Correspondence Address

Applicants note that the pending Office Action was not mailed to the correspondence address of record. In accordance with the Notice of Change of Correspondence Address filed on October 31, 2001 (copy enclosed), all correspondence for the subject application should be sent to:

Customer No. 021005
Hamilton, Brook, Smith and Reynolds, P.C.
530 Virginia Road
P.O. Box 9133
Concord, MA 01742-9133.

Information Disclosure Statement

An Information Disclosure Statement (IDS) was filed on May 25, 2001. To date, Applicants have not received a copy of the Form PTO-1449 initialed by the Examiner to indicate consideration of the cited references. Applicants respectfully request that the Examiner enter the IDS in the record and return a copy of the initialed Form PTO-1449 with the next communication.

Rejection of Claims 1-17 Under 35 U.S.C. § 103(a)

Claims 1-17 are rejected under 35 U.S.C. § 103(a) as being obvious over Earhart, *et al.* (Reference A), in view of McGall, *et al.* (Reference B).

The Examiner states that Earhart, *et al.*, provides a method for synthesizing oligonucleotides within the elements of a molecular array, where a phosphite triester group is oxidized by the addition of iodine in THF, pyridine and water to form a phosphotriester group. The Examiner characterizes the range of iodine concentrations recited in Claims 1-17 as being mere optimization and states that Applicants have not provided evidence of unexpected results. The Examiner previously stated that McGall, *et al.*, teach photolabile protecting groups for use in oligonucleotide synthesis.

Applicants state in the specification at page 19, line 26 to page 20, line 2 that earlier methods of oxidation used 0.1 M solutions of iodine. The instant application teaches that significantly lower amounts of iodine can surprisingly be used to accomplish oxidation to provide arrays with improved sensitivity and functional performance. Specifically, Applicants

teach iodine solutions having concentrations from about 0.005 M to about 0.05 M, and preferably 0.02 M.

A person of ordinary skill in the art would not have been motivated to perform the oxidation with a solution having a dramatically lower concentration of iodine as compared to the prior art. One would typically expect that using a lower concentration of iodine would result in an array with reduced sensitivity and functional performance as a result of incomplete oxidation of phosphite groups (e.g., lower yield of product). Moreover, one of ordinary skill in the art would not have been motivated to reduce the concentration of iodine in the oxidation step because the concentration was not recognized as a result-effective variable (assuming a molar excess of iodine was present).

The MPEP provides a description of a result-effective variable and the relationship between result-effective variables and routine experimentation:

> A particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation. *In re Antonie*, 559 F.2d 618, 195 USPQ 6 (CCPA 1977) (MPEP § 2144.05 (II)(B)).

One of the specific results recognized by the present invention as being affected by the iodine concentration is an oxidation in a nucleic acid away from the phosphite/phosphotriester group, i.e., an unwanted oxidation. The application teaches at page 7, lines 16-17, that using dilute solutions of iodine minimizes oxidations that might be detrimental to portions of the nucleic acid array. Neither Earhart, *et al.* nor McGall, *et al.*, recognized that excessive amounts of iodine resulted in a decreased yield of the desired nucleic acid. That is, neither of the cited references recognized that excess iodine would damage a nucleic acid array. The cited references do not teach or suggest that a reduced concentration of iodine can be used to improve the synthesis of a nucleic acid array.

Applicants have supplied a working example that demonstrates the substantial improvements in functional performance when a nucleic acid array was prepared with a reduced iodine concentration. Example 1 shows the superior assay sensitivity and functional performance, as measured by signal, background and detection, achieved using arrays prepared with a 0.02 M iodine oxidizing solution as compared to arrays prepared with a 0.1 M iodine oxidizing solution. Table 1 shows that, on average, the percentage of 1.5 pM and 3 pM spikes detected by the array prepared with the 0.02 M iodine solution was higher than the percentage of

spikes detected by the array prepared with the 0.1 M iodine solution. This example provides clear evidence that performing the oxidation with substantially lower concentrations of iodine (0.005 M - 0.05 M versus 0.1 M) is *critical* to the invention.

In summary, neither Earhart, *et al.*, nor McGall, *et al.*, teach or suggest the preparation of a nucleic acid array with any specific concentration of iodine. Applicants have discovered that the concentration of iodine used in nucleic acid array preparation affects the sensitivity and functional performance of the array, such that decreasing the concentration of iodine by a concentration of 2-fold (as compared to the prior art) results in an array with enhanced sensitivity and functional performance. Because it was not previously recognized that excess iodine results in damage to a nucleic acid array, adjusting the concentration of iodine to avoid oxidative damage is not routine experimentation and the claimed invention is not obvious over the cited art. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claim 5 Under 35 U.S.C. § 112, First Paragraph

Claim 5 is rejected under 35 U.S.C. § 112, first paragraph, for lack of adequate written description. The Examiner states that the recitation of "PR" for a phosphoramidite group makes it appear that the phosphoramidite group further comprises an "R" group.

Applicants have amended Claim 5 to replace "PR" with "phosphoramidite group." This phrase is supported in Claim 5, as filed, and is clear as to the claimed subject matter. This amendment is non-limiting and does not narrow the scope of the claim in any way; rather, the amendment merely clarifies the nature of the claimed subject matter. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If

the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Lisa M. Treannie

Lisa M. Treannie
Registration No. 41,368
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

3. (Twice Amended) A method in accordance with claim 2, wherein said synthesizing comprises the sequential steps of:

a) removing a photoremovable protecting group from at least a first area of a surface of a substrate, said surface comprising immobilized nucleotides on said surface, said nucleotides capped with a photoremovable protecting [protective] group, without removing a photoremovable [photoremoveable] protecting group from at least a second area of said surface;

b) simultaneously contacting said first area and said second area of said surface with a first nucleotide to couple said first nucleotide to said immobilized nucleotides in said first area, and not in said second area, said first nucleotide capped with said photoremovable protecting [protective] group;

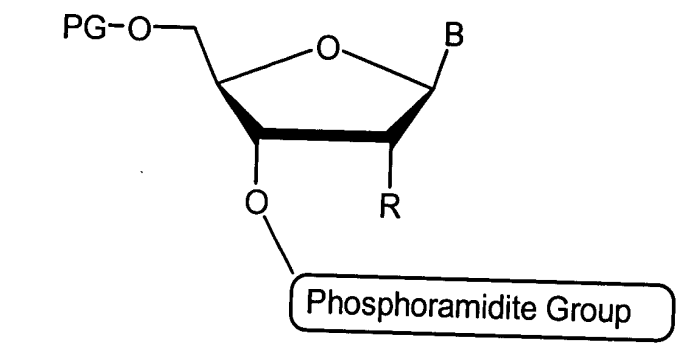
c) removing a photoremovable [photoremoveable] protecting group from at least a part of said first area of said surface and at least a part of said second area;

d) simultaneously contacting said first area and said second area of said surface with a second nucleotide to couple said second nucleotide to said immobilized nucleotides in at least a part of said first area and at least a part of said second area;

e) performing additional removing and nucleotide contacting and coupling steps so that a matrix array of at least 100 nucleic acids having different sequences is formed on said support;

with the proviso that the coupling steps further comprise oxidizing an initially formed phosphite ester linkage to a phosphate ester linkage using from about 0.005 M to about 0.05 M iodine in an aqueous solvent mixture.

5. (Twice Amended) A method in accordance with Claim 3, wherein said nucleotides have the formula:



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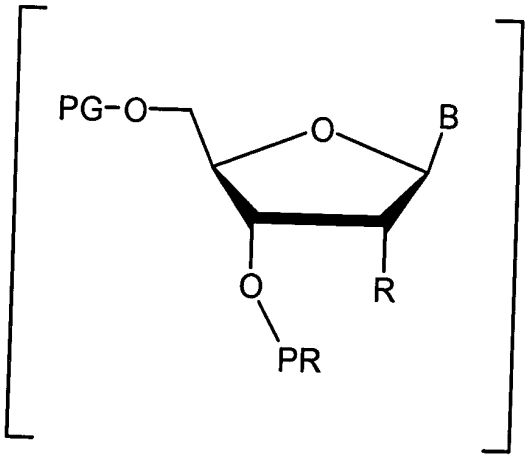

wherein

B is a member selected from the group consisting of natural or unnatural adenine, natural or unnatural guanine, natural or unnatural thymine, natural or unnatural cytosine, and natural or unnatural uracil;

R is a member selected from the group consisting of hydrogen, hydroxy, protected hydroxy, halogen and alkoxy;

[PR is a phosphoramdite group;] and

PG is a photoremovable protecting [photoremoveable protected] group.

17. (Amended) A method in accordance with Claim 5, wherein B is selected from the group consisting of adenine, guanine, cytosine and thymine, R is hydrogen, PG is MeNPOC, the phosphoramidite group [P] is $-P(OCH_2CH_2CN)N(iPr)_2$ and said solution is about 0.02 M iodine in a mixture of water, pyridine and THF.